ORIGINAL ARTICLE

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Development of biscuits with viscera of bullfrog (*Lithobates catesbeianus*) for adult dogs

*Desenvolvimento de biscoitos com vísceras de rã-touro (*Lithobates catesbeianus*) para cães adultos*

Ana Alice Santos Cortez Leigue[1], Jerônimo Vieira Dantas Filho[2,3]*,
Donovan Filipe Henrique Pinto[1], Jucilene Braitenbach Cavali[1], Rute Bianchini Pontuschka[1]

ABSTRACT: The study aimed to develop dog biscuits using bullfrog (*Lithobates catesbeianus*) viscera. After sterilizing the viscera, a pâté was produced and combined with lard, wheat flour, chemical yeast, and water to make biscuits. Microbiological safety was ensured by analyzing total mesophilic aerobes, molds, and yeasts through colony counts. The chemical composition of the biscuits (moisture, crude protein, ether extract, crude fiber, and ash) was determined. A sensory analysis, known as a preference test, was conducted where dogs were offered both the bullfrog biscuits and a well-accepted commercial brand to evaluate their preferences based on First Choice, First Consumption, and Reason for Ingestion. The commercial biscuits also underwent microbiological analysis. Results showed that both the commercial and bullfrog biscuits met international standards for mesophilic aerobes, molds, and yeasts. The proximate composition of the bullfrog biscuits was: moisture (4.52%), crude protein (14.6%), ether extract (10%), carbohydrates (68.26%), crude fiber (0.01%), and ash (1.55%), with a caloric value of 421.66 kcal per 100g. Sensory analysis revealed no significant difference in First Choice and Intake Ratio. However, dogs preferred the bullfrog biscuits for First Consumption (64.92%) compared to the commercial biscuits (35.02%). Despite the notable preference for First Consumption, no significant differences were observed for other sensory variables, suggesting that the bullfrog biscuits were well accepted by the dogs and had the potential to be a marketable product.

KEYWORDS: bullfrog farm; sensory analysis; pet food; use of waste.

RESUMO: O estudo desenvolveu biscoitos para cães adultos utilizando vísceras de rã-touro (*Lithobates catesbeianus*). As vísceras foram esterilizadas e transformadas em patê, que foi combinado com banha suína, farinha de trigo, fermento químico e água para elaborar os biscoitos. A segurança microbiológica foi garantida pela análise de aeróbios mesófilos totais, bolores e leveduras, por meio da contagem de colônias em placas de Petri. A composição química dos biscoitos incluiu umidade, proteína bruta, extrato etéreo, fibra bruta e cinzas. Um teste de preferência sensorial foi realizado, oferecendo os biscoitos de rã aos cães junto com biscoitos comerciais. O teste avaliou Primeira Escolha, Primeiro Consumo e Razão de Ingestão. Os biscoitos comerciais também passaram por análise microbiológica. Os resultados mostraram que ambos os tipos de biscoitos estavam dentro dos limites internacionais para contagem de aeróbios mesófilos, bolores e leveduras. A composição dos biscoitos de rã foi: umidade (4,52%), proteína (14,6%), extrato etéreo (10%), carboidratos (68,26%), fibra bruta (0,01%) e cinzas (1,55%), com valor calórico de 421,66 kcal/100g. A análise sensorial não indicou diferença significativa para Primeira Escolha e Razão de Ingestão. No entanto, o Primeiro Consumo mostrou uma preferência dos cães pelos biscoitos de rã-touro (64,92%) em relação aos comerciais (35,02%). Embora tenha havido preferência considerável pelo biscoito de rã-touro no Primeiro Consumo, não houve diferença para as demais variáveis sensoriais, indicando boa aceitação dos biscoitos de rã-touro pelos cães, com potencial para se tornarem um produto vendável.

PALAVRAS-CHAVE: alimentos para animais de estimação; análise sensorial; aproveitamento de resíduos; ranicultura.

[1]Dept. de Engenharia de Pesca, Universidade Federal de Rondônia, Presidente Médici/RO, Brazil
[2]Dept. de Medicina Veterinária, Centro Universitário São Lucas Ji-Paraná Afya, Ji-Paraná/RO, Brazil
[3]Programa de Pós-Graduação em Ciências Ambientais, Universidade Federal de Rondônia, Rolim de Moura/RO, Brazil
*Corresponding author: jeronimo.filho@saolucasjiparana.edu.br
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INTRODUCTION

In Brazil, the cultivation of bullfrogs (*Lithobates castebeianus* Shaw, 1802) began in confinement systems, especially in the system called amphigrange, which despite being primitive, provides the basic conditions for the animals to feed, develop, mate and protect themselves satisfactorily (Oliveira, 2015). Over the years, cultivation has faced problems linked to the reduction in production units, exports, production flow and the lack of a technological package for bullfrog cultivation (Lima; Agostinho, 1989; Oliveira, 2015).

That's why, most bullfrog farming is characterized by small, low-tech crops, with family labor. Normally, it is carried out as a secondary activity, just to supplement income, and is not seen as a promising and profitable business (Corrêa *et al.*, 2008; Sousa; Maltarolo, 2019; Afonso, 2012). Despite these problems faced since the implementation of bullfrog cultivation in Brazil, there was growth in the segment, which increased its production by more than 2600% between years 1989 and 2001, according to the Food and Agriculture Organization (FAO). Production reached 129,329 tons in year 2017, according to the latest agricultural census (IBGE, 2017).

During the bullfrog raised period, these animals go through different stages of development that involve different costs depending on the demand for food, space and water (Tokur; Gurbuz; Ozyurt, 2008). The activity is costly, since the carcass yield can vary depending on the cultivation method, being 46.29% in a flooded cultivation system and 51.46% for bullfrog farm, in addition to the influence of the animal's sex, so that females provide lower yield, possibly due to the development of gonads for reproduction, another factor that also influences carcass yield is the amount of crude protein present in the feed (Nascimento *et al.*, 2019; Cribb; Afonso; Ferreira, 2013). Therefore, almost 50% of the animal's body is waste such as skin, viscera, head and paws, which makes the use of this organic matter interesting. In the current overview, we have almost total use of slaughter residues from different types of animal farming, such as cattle farming, with full use of the animal's carcass: bones, tallow, manure, leather, viscera (Jayathilakan *et al.*, 2012). We can also cite as an example of total use of by-products the poultry farming activity, which generates different waste after slaughter: feathers, feet, fat, viscera, bones and cartilage (Ferreira, 2020).

The same use is not applied to bullfrog slaughter waste, as there are few studies that address the use of waste, especially the viscera, which constitute a significant part of the material. That's why, a reuse destination is necessary, both to minimize the effects on the environment, where these residues are discarded by some producers, and to add value to organic material that would otherwise be discarded. Furthermore, reuse can help supplement the farmer's income, as we have seen, it is mainly family labor.

A possible way of using frog farming waste would be in the pet food factories, which has a promising market. According to the Brazilian Franchising Association (2021), this national segment is considered one of the top five in the world. According to the Market Information Commission (Coinf) of the National Union of the Animal Health Products Industry (Sindan), the forecast is for an increase of 15.8% in the pet segment in year 2023, and 15% in year 2024.

The segment of pet food factories alone had revenues of R$33.1 billion in the first quarter of 2022 (Haddad *et al.*, 2022). This growth in the pet food industry is since owners are increasingly seeking to offer better quality food for their animals. Furthermore, the pet food market uses a large part of animal slaughter waste, mainly from cattle and poultry, in the form of bone meal, blood meal and even feather meal (Haddad *et al.*, 2022). These flours are used in the manufacture of complete dry or wet foods and snacks for pets.

Biscuits, called "specific foods" by Brazilian legislation (Brasil, 2009), emerged with the purpose of pleasing or contributing to the education of dogs and cats, and have gained space in the pet market. They are found in the form of biscuits, cookies, snacks, and steaks. Despite being nutritious, they are not complete foods like feed, and their consumption should not exceed 10% of the animals' caloric needs (NRC, 2006). The label must also contain information about the maximum amount to be offered daily depending on the animal's weight, in addition to containing the message "This product does not replace the complete food" (Brasil, 2009).

Using contextualization and assumptions, the study aimed to develop biscuits for adult dogs through the use viscera of bullfrog (*Lithobates catesbeianus*).

MATERIAL AND METHODS

Obtaining bullfrog viscera

This research with bullfrogs did not need to be evaluated by an Animal Ethics Committee because the bullfrog viscera were donated by a bullfrog farm. The bullfrog viscera were purchased from a bullfrog farm that also has a slaughter unit, both located in the city of Presidente Médici, Rondônia state, with a Municipal Inspection Seal (SIM). The bullfrog cuts commercialized by the slaughter unit are "thighs" and "frog and bird". Typically, the owners of this frog farm dispose of processing waste along with the property's trash. The viscera obtained were transported in a thermal box to the Laboratório de Análise de Alimentos, at the Universidade Federal de Rondônia (UNIR), where they were frozen at -18°C until use. As for the mixing ingredient, wheat flour, lard and chemical baking powder were obtained from local markets.

Development of biscuits

The bullfrog viscera were thawed in the refrigerator, weighed and sterilized in an autoclave for 15 min at 121°C. After cooling, the viscera were drained through a stainless-steel sieve to remove excess liquid and weighed again. Finally, they were homogenized with a Walita household mixer, with 400 watts of power. The remaining ingredients were added and mixed until a uniform dough was obtained with an ideal texture for rolling out with a rolling pin (without sticking to the work surface). Subsequently, the biscuits were cut with a round mold, 0.5 cm thick, placed on baking trays and placed in the oven at 290°C for 30 minutes, being inverted halfway through the time to bake evenly (Figure 1). After baking and cooling, the biscuits were weighed to obtain the yield.

Microbiological analysis

The methodology used was surface plating, described by Silva *et al.* (2007). Serial dilutions were prepared for both the bullfrog and commercial (control) biscuits. Dilutions were inoculated in duplicate on Nutrient Agar medium. Inoculation took place at 35°C for 48 hours. Afterwards, the colonies were counted and calculated to find the number of Colony Forming Units g^{-1} (CFU g^{-1}) (Silva *et al.*, 2007).

Regarding the analysis of molds and yeasts, after serial dilution, the potato glucose agar culture medium acidified with 10% Tartaric acid (Silva *et al.*, 2007) was used, plating on the surface. This method is based on verifying the ability of these microorganisms to develop in culture media with a pH close to 3.5 and an incubation temperature of 25°C. The use of acidified media selectively promotes the growth of fungi, inhibiting most bacteria present in the food. Incubation occurred at 25.0 ±1.0°C, for 5 to 7 days, and after this time the colonies were counted.

Figure 1. (A) homogenization of bullfrog viscera; (B) weighing and separating ingredients; (C) opening the dough and cutting the biscuits; (D) cut biscuits ready to be baked.

Proximate composition and texture analysis

The moisture, crude protein, ether extract, crude fiber and ash contents were determined in duplicate according to the Adolfo Lutz Institute protocols (IAL, 2008). Carbohydrates were estimated by difference. The total caloric value was obtained by multiplying the average values of protein, lipids and carbohydrates by factors 4, 9 and 4, respectively (Souci; Fachman; Kraut, 2000).

The methodology for determining the instrumental texture profile was conducted out according to Bourne (2002). The attributes tested were Compression strength (KgF mm^{-2}), Hardness (N), Adhesiveness (mm^2), Fracturability (N), Chewiness (N mm^{-1}) and Spreadability (N mm^{-2}) (Sogabe *et al.*, 2021), considering the shear stress and viscosity of water at 26°C. These attributes were analyzed on a TAXT.plus texturometer, using Exponent Stable Micro Systems software (Stable Micro System Ltd, Vienna Court, United Kingdom) (Borges *et al.*, 2013).

A total of 6 were randomly selected from each feed sample. In the texturometer analyses, they were positioned vertically and horizontally on a platform. A cylindrical probe with a flat end, ½ inch in diameter, was used. Regarding the conditions of the instrumental texture tests, the pre-test speed was 2 mm s^{-1}, post-test 10 mm s^{-1}, distance 4.0 mm (Sogabe *et al.*, 2021).

Dog preference tests

The food preference test was carried out based on the methodology of Zanatta *et al.* (2016), and the experimental protocol was approved by the Animal Use Ethics Committee, with protocol no. 013/2020. A total of 19 adult dogs of varying sizes, males and females, healthy and selective (they smell before eating food) were chosen. Of these, seven were domiciled in the municipality of Ji-Paraná; eight were sheltered at the non-governmental organization Amparo Animal, based in Ji-Paraná city, and four were community dogs, under the care of the UNIR Campus community in Presidente Médici city.

The biscuits were provided to each animal individually one hour after the meal. The biscuits of each type were offered simultaneously, the bullfrog biscuits in one feeder and the commercial biscuit in another. The feeders were made of plastic, identical and the total amount of biscuits offered respected the limit of 10% of caloric needs. The tests were carried out during 5 different days of attempts (quintuplicate), totaling 95 observations. The positions of the feeders were reversed for each test, avoiding laterality. Thus, in this sensory analysis, the criteria to consider the favorite biscuit were: First Choice; First Consumption and Intake Reason.

The First Choice test was conducted out using the double choice method (Solà-Oriol; Roura; Torrallardona, 2009). In the test, it was recorded which of the two feeders the animal approached first, regardless of whether it had been consumed or not. The First Consumption corresponded to the biscuit that was completely consumed first. The Intake Ratio (IR) of the biscuits in the current study were obtained by Equation (1) according to Carciofi (2008).

$$IR(\%) = M_{ing}\ (g)\ /\ M_{total}\ (g) \quad (1)$$

Where, Intake Ratio (%) = [mass (grams) of bullfrog or commercial biscuit ingested / total mass (grams) consumed (bullfrog + commercial)] x 100.

Experimental design and statistical analyzes

The study was conducted in a completely randomized design, to compare the averages of microbiological data, proximate composition and label of the commercial biscuit (control) and those of the sensory analysis, the Student's t-test was applied ($p < 0.05$). To compare the averages of the proximate composition in bullfrog biscuit in relation to the guarantee levels on the labels of other commercial biscuits, the Tukey's test was applied ($p < 0.05$). Finally, in addition to the Student's t-test for sensory analysis, a polynomial regression was conducted out to verify the dogs' preference and intake percentages.

All statistical analyzes were performed using RStudio Development Core Team, version 3.5.3.

RESULTS

There was no growth of total mesophilic aerobi microorganisms in the commercial biscuit sample (control), and the values for the bullfrog biscuit were below the international limit, indicating that both were suitable for animal consumption in terms of this aspect of quality. Regarding the molds and yeasts, the two biscuit varieties showed values below the recommended range (Table 1).

Regarding the proximate composition, its variables showed a statistical difference ($p < 0.05$) between the bullfrog biscuits and the control. Remembering that the composition data for the commercial biscuits (average of 4 different brands) were obtained directly from the packaging. Moisture showed a higher value ($p < 0.05$) in the commercial biscuits (control) compared to the bullfrog biscuits. Because water is closely linked to the development of microorganisms in food, a low moisture content is desirable to obtain longer shelf life (Table 2).

Crude protein expressed a higher value ($p < 0.05$) for bullfrog compared to commercial protein (control) biscuits, 14.6 and 10.0%, respectively. In the case of snacks, the palatability that animal protein gives to the product is important, as it is essential that it is well accepted by the animal so that it fulfills its purpose. Bullfrog biscuits had the highest ($p < 0.05$) averages of crude protein (14.6%), etheral extract (10%), carbohydrates (68.26%) and caloric value (421.66%) compared to commercial biscuits (Table 2).

According to statistical analyses, the moisture content of the bullfrog biscuit was lower ($p > 0.05$) than that of commercial biscuits. The crude protein, carbohydrates and caloric value of bullfrog biscuits were higher ($p < 0.05$) compared to commercial biscuits. However, the averages of mineral matter and fibrous matter were lower ($p < 0.05$) in relation to commercial biscuits. As observed, the protein values of the bullfrog biscuit were higher in relation to commercial brands, including the control, except for the PR biscuit, which had 15% CP. Lipids and carbohydrates were also higher for bullfrog compared to other commercial and control biscuits. In general, bullfrog biscuits showed rates similar to or higher than commonly commercialized biscuits (Table 3).

Regarding the texture attributes, bullfrog biscuits (6254.14 KgF mm^{-2}), FT (5089.93 KgF mm^{-2}) and PR (4893.95 KgF mm^{-2}) showed the highest compression strength averages to be fractured ($p < 0.05$), while biscuits FT (16917.03 N), BW (18605.94 N) and PU (17158.42 N)

Table 1. Analysis of total aerobic mesophiles, molds and yeasts from biscuits, expressed in log CFU g^{-1}.

Biscuits	Aerobic mesophiles	Molds and yeasts
	Log UFC g^{-1}	Log UFC g^{-1}
Bullfrog	2.30ᵃ	2.17ᵃ
Commercial (control)	<10* ᵇ	2.17ᵃ
p value	0.001	0.015

CFU g^{-1}: Colony forming units per gram *<10 log CFU g^{-1} – Absence of growth. Different letters (a,b) between the lines: values are different from each other, according to the Student's t-test ($p < 0.05$).

Table 2. Means and standard deviations of the proximate composition (%) and caloric value (kcal $100g^{-1}$) in bullfrog and commercial (control) biscuits.

Variables	Biscuits		*p* value
	Bullfrogs	Commercial (control)*	
Moisture	4.52 ± 0.03ᵇ	10.00 ± 0.6ᵃ	0.0233
Crude protein	14.6 ± 0.40ᵃ	10.00 ± 0.4ᵇ	0.0370
Ethereal extract	10.00 ± 0.10ᵃ	5.50 ± 0.50ᵇ	0.0111
Carbohydrates**	68.26 ± 0.40ᵃ	63.50 ± 0.38ᵇ	0.0214
Ash	1.55 ± 0.10ᵇ	7.00 ± 0.45ᵃ	0.0090
Raw fiber	0.01 ±0.00ᵇ	4.00 ± 0.20ᵃ	0.0127
Caloric value	421.66 ± 3.90ᵃ	350.00 ± 3.24ᵇ	0.0393

Data for bullfrog biscuits showed as mean percentage ± standard deviation of duplicate analyses; *Values declared on the label; **Calculated by difference; if there are different letters (a, b) between the columns it is different according to the Student's t-test ($p < 0.05$).

Table 3. Proximate composition values (%) of bullfrog and commercial (control) biscuits and guarantee levels (%) expressed on the labels of the comercial brands (control), FT, BW, PU, and PR.

Parameters	Proximate analysis	Warranty levels				
	Bullfrog	Controle (comercial)	FT	BW	PU	PR
Moisture (max.)	4.52 ± 0.03[b]	10.00 ± 0.60[a]	10.00 ± 0.60[a]	10.00 ± 0.60[a]	10.00 ± 0.60[a]	10.00 ± 0.60[a]
Crude protein (min.)	14.6 ± 0.40[a]	10.00 ± 0.40[b]	12.00 ± 0.50[ab]	12.00 ± 0.50[ab]	10.00 ± 0.40[b]	15.00 ± 0.60[a]
Mineral matter (max.)	1.55 ± 0.10[c]	7.00 ± 0.45[a]	7.00 ± 0.45[a]	7.00 ± 0.45[a]	5.50 ± 0.35[ab]	3.00 ± 0.19[bc]
Fibrous matter (max.)	0.01 ± 0.00[c]	4.00 ± 0.20[b]	1.50 ± 0.08[bc]	1.50 ± 0.08[bc]	5.50 ± 0.28[a]	2.00 ± 0.10[ab]
Ethereal extract (min.)	10.00 ± 0.10[b]	5.50 ± 0.50[c]	8.00 ± 0.72[ab]	8.00 ± 0.72[ab]	5.00 ± 0.45[c]	13.00 ± 1.17[a]
Carbohydrates	68.26 ± 0.40[a]	63.50 ± 0.38[b]	61.50 ± 0.36[ab]	61.50 ± 0.36[ab]	64.00 ± 0.38[b]	57.00 ± 0.34[c]
Caloric value (kcal $100g^{-1}$)	421.66 ± 3.90[a]	350.00 ± 3.26[bc]	366.00 ± 3.40[b]	366.00 ± 3.40[b]	341.00 ± 3.17[c]	405.00 ± 3.77[ab]

If there are different letters (a,b,c) between the columns, it is different according to the Tukey's test (p < 0.05).

Table 4. Compression strength, hardness and adhesiveness of bullfrog and comercial brands (control) biscuits, FT, BW, PU, and PR.

Biscuits	Texture attributes		
	Compression strength (KgF mm^{-2})	Hardness (N)	Adhesiveness (mm^2)
Bullfrog	6254.14 ± 859.32[a]	11120.59 ± 1745.93[b]	-4,34 ± 0.62[b]
FT	5089.93 ± 629.11[a]	16917.03 ± 2178.91[a]	-6.17 ± 0.89[a]
BW	2454.69 ± 303.40[c]	18605.94 ± 4089.59[a]	-6.91 ± 1.54[a]
PU	3937.80 ± 804.49[b]	17158.42 ± 4365.10[a]	-5.04 ± 0.96[b]
PR	4893.95 ± 1364.92[a]	13619.50 ± 3357.21[ab]	-5.65 ± 0.96[b]
p value	0.023	0.037	0.018

If there are different letters (a,b,c) between the columns, it is different according to the Tukey's test (p < 0.05).

showed the highest hardness averages ($p < 0.05$). In addition to these attributes, the FT (-6.17) and BW (-6.91) biscuits showed the highest stickiness averages ($p < 0.05$) (Table 4).

Bullfrog biscuits, FT, PU and PR showed the lowest means of fracturability, chewiness and spreadability ($p < 0.05$). In other words, while the BW biscuit showed lower quality for these attributes, the bullfrog biscuit showed a similar textural quality to other biscuits available on the market (Table 5).

In the regression analysis showed in Figure 2 it can be seen that despite the higher compression strength required, the bullfrog biscuit is not as hard as the average commercial biscuit. In fact, it showed better adhesiveness, chewability even with lower fracturability and spreadability ($p < 0.05$) (Table 4 and Table 5).

As for the sensory analysis, the First Choice variable did not express a statistical difference ($p > 0.05$) for the two types of biscuit, as well as the Intake Ratio, since the animals consumed both types completely. However, First Consumption indicated a difference ($p < 0.05$) between bullfrog and commercial (control), 64.92 and 35.08%, respectively (Table 6), indicating preference for the bullfrog biscuit.

Figure (3 A) confirms that there was no preference for First Choice ($p^2 = 0.47$). However, there was variation for First Consumption (Figure 2 B), the results of which indicate preference ($p^2 = 0.78$) in comparison to the biscuit commonly commercialized on the market.

Table 5. Fracturability, chewiness and spreadability of of bullfrog and comercial brands (control) biscuits, FT, BW, PU, and PR.

Biscuits	Texture attributes		
	Fracturability (N)	Chewiness (N mm^{-1})	Spreadability (N mm^{-2})
Bullfrog	2122.83 ± 290.83[b]	1.05 ± 0.16[b]	0.90 ± 0.13[b]
FT	2606.12 ± 357.04[b]	1.33 ± 0.21[b]	0.88 ± 0.13[b]
BW	4330.98 ± 593.34[a]	3.88 ± 0.61[a]	2.77 ± 0.40[a]
PU	2544.48 ± 348.59[b]	1.12 ± 0.18[b]	1.02 ± 0.14[b]
PR	2622.19 ± 359.24[b]	1.31 ± 0.21[b]	1.10 ± 0.16[b]
p value	0.037	0.031	0.029

If there are different letters (a,b,c) between the columns, it is different according to the Tukey's test (p < 0.05).

DISCUSSION

To reach an ideal proportion of ingredients for the biscuit dough, previous tests were carried out with different amounts of each component, including rice flour replacing wheat flour,

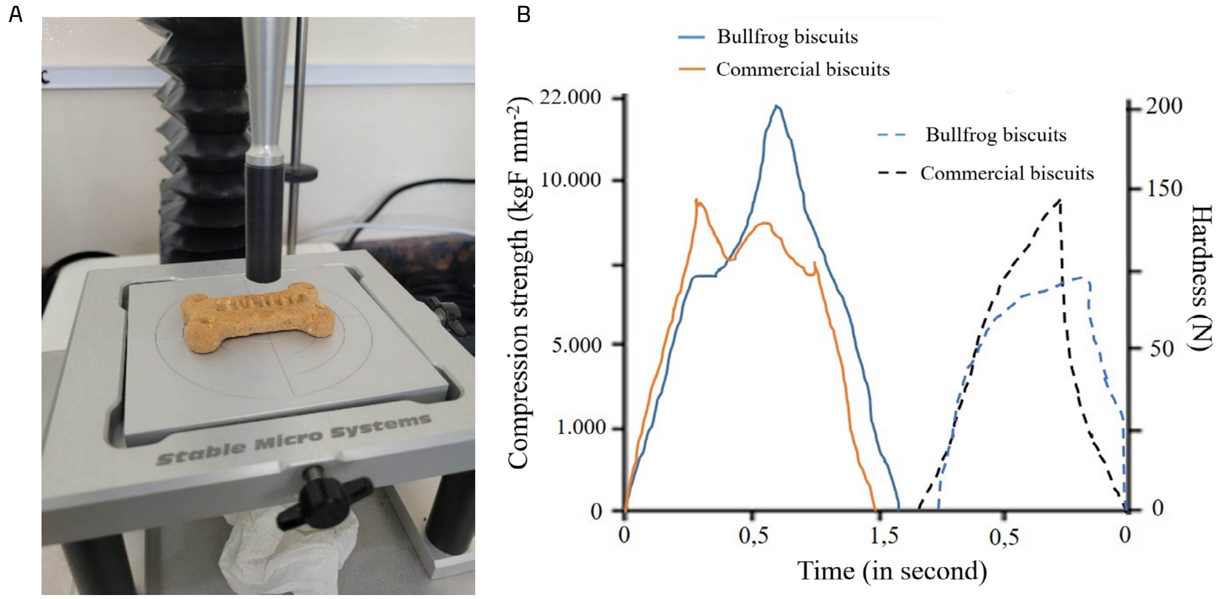

Figure 2. Instrumental texture analysis (A) and polynomial regression analysis of the compression strength and hardness of bullfrog and commercial biscuits (B).

Table 6. Percentage (%) of First Choice, First Consumption and Intake Ratio.

Biscuits	Percentages (%)		
	First Choice	First Consumption	Intake Ratio
Bullfrog	51.61 ± 23.70[a]	64.92 ± 7.30[a]	50 ± 0.00[a]
Commercial (control)	48.39 ± 22.22[a]	35.08 ± 3.94[b]	50 ± 0.00[a]
p value	0.0630	0.0282	0.1000

Data showed as mean percentage ± standard deviation of quintuplicate analyses; if there are different letters (a,b) between the lines it is different according to the Student's t-test ($p < 0.05$).

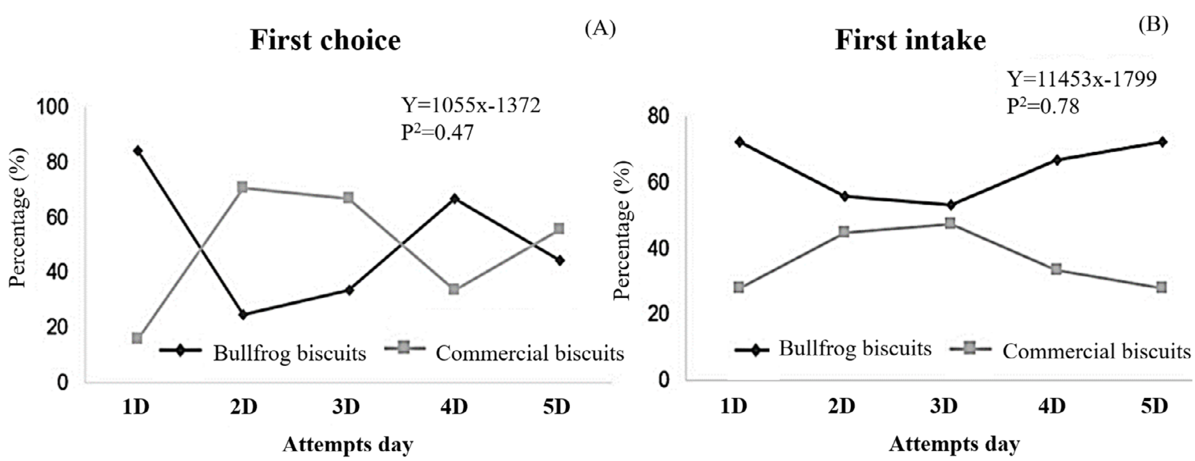

Figure 3. Polynomial regression analyzes for the percentages of First Choice and First Consumption.

with the aim of making the biscuit gluten-free, however, the result was not satisfactory, resulting in a crumbly dough that was difficult to work with.

As in Brazil there are still no official criteria regarding the microbiological quality of food for dogs, the CFU g^{-1} results obtained for mesophiles were compared to international recommendations for this group (Andriguetto *et al.*, 1998), with the product having to present values below 6 log CFU g^{-1} or 106 CFU g^{-1}. Likewise, there are no national legal standards for fungi in those foods. The international recommendation is that levels should not exceed 4 log CFU g^{-1} or 104 CFU g^{-1} (GMP, 2008). According to these standards, it appears that both cookies were within the recommended microbiological limits (Table 1).

The numbers obtained in the microbiological analysis are optimistic, since a high number of microorganisms causes changes in odor, flavor, texture, among others, in addition to the potential danger to the health of animals when it comes to pathogenic microorganisms (Franco, 2008). In the case of fungi, it is noteworthy that the presence of mycotoxins is highly harmful, even causing death (Terada-Nascimento *et al.*, 2023). However, during the entire processing phase of the bullfrog biscuit, good manufacturing practices were employed: hand hygiene; use of gloves when relevant; constant use of a mask; cleaning the countertop, containers, instruments and cutlery. These procedures helped to obtain biscuits with good microbiological quality.

Poppi *et al.* (2023) found satisfactory results when analyzing biscuits made with fish flour, finding values <10 MPN g^{-1} for coliforms at 35 and 45°C; 0.01 Log CFU g^{-1} for *Staphylococcus coagulase* and absence of *Salmonella sp*., which indicates that the biscuits were also safe for consumption by dogs. The same authors agreed on the importance of low moisture in relation to shelf life. Its dog biscuits made with flour from different fish species had 5.80 to 6.21% moisture. Vecchiato *et al.* (2022) commented in his research on dog food regarding the importance of food moisture in terms of animal dryness, as food with low moisture values makes feces drier. However, as dog treats do not fall into the category of complete food, although are foods for pleasure or reward and should be provided to the animal in small daily quantities, it is therefore unlikely that they will cause any problems with the animal's intestinal health due to the low moisture.

In the same way that the crude protein showed a higher value than the commercial biscuit, the etheral extract (lipids) was found to be higher in the bullfrog compared to the commercial (control) biscuits, possibly due to the addition of pork fat in the dough, which suggests that this ingredient should be better balanced in relation to the others to reduce the caloric value. Despite the high results for the etheral extract, consumption of the bullfrog biscuit would not cause obesity in the animal, given the recommended daily intake is met. Lipids even increase the palatability of the food (Wortinger, 2009).

When evaluating dogs' preference for meat and vegan biscuits, he reported that there was a greater consumption of meat, since they had a higher percentage of crude protein and ether extract. The author understands that these two items had a considerable influence on palatability (Knight; Satchel, 2021). Similar results were achieved by Lei *et al.* (2019) in his study regarding the acceptability of dog biscuits containing Black Soldier fly (*Hermetia illucens*) larvae flour. The crude protein and lipid values of the product were higher than that of the biscuit used as a control in their study, with crude protein (18.99%) and ethereal extract (5.20%) in larvae flour and crude protein (10.57%) and ethereal extract (4.20%) in commercial biscuit. Regarding the caloric value, the high index of the bullfrog biscuit is due to the addition of pork lard to the biscuit dough together with carbohydrate (wheat flour), which was the component in greatest quantity in the composition of the biscuits.

Crude fiber showed a higher value for control (commercial) compared to frog fiber. Fiber in dog food regulates intestinal motility (Vecchiato *et al.*, 2022). For commercial feeds, a minimum of 6% fiber is recommended (Andriguetto *et al.*, 1998). However, as bullforg biscuits are not a complete food, the fiber content in their composition should not interfere with the animal's health when offered in the recommended amount. Chaves (2022), implemented industrial waste flour from acerola processing in the production of dog treats. The proportion of crude fiber in the product was 14.16%. Nevertheless, ash (total minerals) expressed a higher value for commercial biscuits in relation to bullfrog biscuit, 7.00 and 1.55%, respectively. When dealing with ash, the parameters are to establish the maximum mineral matter that a food can contain instead of the minimum, as its excess causes harmful interactions with other nutrients (Case; Carey; Hirakawa, 1998).

The carbohydrate values calculated by difference were 68.26% for the bullfrog biscuit and 63.5% for the commercial biscuits. Despite being statistically different, the values are close, which may indicate that the bullfrog biscuit would not require a different balance in terms of wheat flour. Carbohydrates have the function of providing volume and structure to the biscuit dough, however, when in excess, they can cause poor acceptance and palatability for dogs (Poppi *et al.*, 2023). There are several factors that influence dogs' food choices, which can be intrinsic (hunger and satiety) or extrinsic, such as the characteristics of the food: ingredients; processing; physical form; temperature; texture etc. (Zanatta *et al.*, 2016).

Regarding the First Choice variable, one of the factors that must be taken into account is the dogs' keen sense of smell, as they may be more attracted to food that emanates a meat odor (Houpt; Smith, 1981). Nonetheless, this interest may only last momentarily, and it is not mandatory for the animal to first consume (First Consumption) the food it first approached (First Choice). Saad *et al.* (2004) point out that dogs have specific food preferences such as beef instead of chicken, animal fat instead of vegetable fat and new foods instead of known foods. The results for First Consumption (first intake in the graph) may come from the higher percentage of crude protein in the bullfrog biscuit and the added pork fat, which contribute to a strong smell and flavor that attract the animal (Felix; Oliveira; Maiorka, 2010).

Knight *et al.* (2022), in their research evaluating the preference between vegan and meat biscuits for dogs, found that although dogs, in their majority (58.7%), approached vegan biscuits first (First Choice), these were not necessarily consumed first (First Consumption), with chicken meat biscuits being the First Consumption preference. Regarding the Intake Ratio, there was no significant difference ($p > 0.05$), as the dogs consumed 100% of both biscuits in all observations. The Intake Ratio is an excellent indicator of animal preference and both products can be considered palatable for dogs. Similarly, in the study of Pires *et al.* (2013) it was observed that the Intake Ratio indicated that different cookies offered had pleased the animals, but the other sensory parameters indicated a difference in preference.

According to Marx *et al.* (2016) the soft texture of the diets promotes the deposition of bacterial plaque, while the dry texture promotes mechanical forces during chewing on the teeth, therefore lower incidence of residue deposition. When comparing bullfrog biscuits with commercial ones, it is noted that despite being resistant to pressure to fracture, it is not rigid enough to make chewing difficult. Emphasizing that because its average adhesiveness is lower, it facilitates swallowing and reduces the amount of residue in the oral cavity, thus being beneficial to the pet's oral health.

Poppi *et al.* (2023) developed biscuits from the inclusion of fish visceral flour (tilapia and salmon) and chicken offal, with the aim of evaluating microbiological and nutritional quality, texture and palatability. According to the authors, the inclusion of flour in pet biscuits did not influence the protein and carbohydrate contents, whose average values were 18.48 and 50.23%, respectively. However, they influenced the moisture content, lipids, ash and caloric value. The microbiological analysis by Poppi *et al.* (2023) indicated that the biscuits had appropriate qualities to be fed to dogs. Regarding texture and acceptability, there was no significant difference between treatments. Regarding acceptability, dogs and cats use their sense of smell, as their taste buds are inferior to that of humans (Bautz, 2013). In the study by Souza *et al.* (2022) in which he used tilapia carcass waste did not obtain acceptance and/or preference from dogs, rejection was gradually proportional to the percentage of fish flour, with the odor being suggested as a contributing factor.

The bullfrog biscuits were accepted in the first and second supply, demonstrating that the product's odor was pleasant. Acceptance in acceptability and palatability tests in the preference assessment is an indicator of the absence of neophobia for the animal, which Carneiro (2017) infers from the occurrence of neophobia and suggests that the product is harmful to health.

CONCLUSIONS

From a microbiological point of view, both bullfrogs and commercial biscuits were in compliance with international legislation, posing no danger to animal consumers. The crude protein, etheral extract and caloric value of the bullfrog biscuits were higher than the commercial ones. In sensory analysis tests, First Choice and Intake Ratio did not shown significant differences between the bullfrog biscuit and the commercial one. However, in the First Consumption there was a considerable preference for the bullfrog biscuit, indicating good acceptance by the animals for whom the product is intended, which would make the product salable.

A future study with an emphasis on market niche would be necessary to implement the production of biscuits and snacks by bullfrog-producing agribusinesses.

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